HPLC Method development and instrument QC for Aldehyde and Ketone compounds

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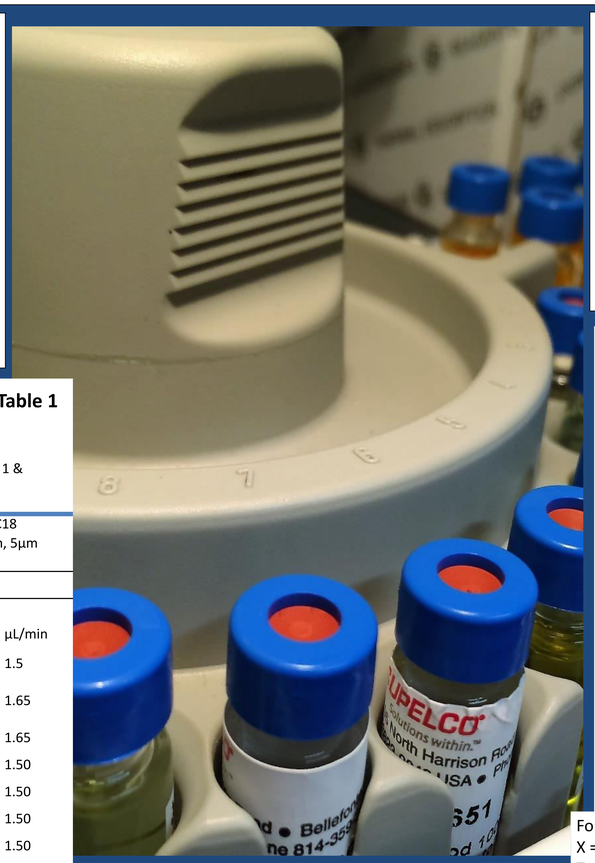
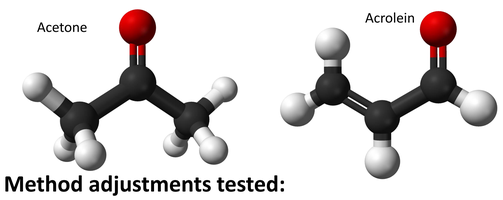
Summary

The Uintah Basin periodically experiences high ozone levels during the winter season (Lyman et al). Carbonyl compounds are produced from various emission sources and are precursors to ground level ozone production. Of these compounds, several aldehydes and ketones are volatile and listed as hazardous under the Clean Air Act (1990). We regularly measure carbonyl concentrations in air via high-performance liquid chromatography (HPLC) as part of our efforts to better understand and improve air quality in the Uintah Basin. For this project, we investigated possible improvements to our HPLC system to increase resolution, identification, and quantification of carbonyls in collected air samples. The goals of this work were to (1) achieve better separation of individual carbonyls to do so we investigated the impact of changes to flow rate/pressure and eluent composition to the method. (2) improve the signal/noise ratio of our measurements. To address the second objective we determined detection limits for the modified instrument configuration by modifying sample injection volumes



Carbonyl Separation

Acrolein and acetone both have three carbons and have similar retention times. And are thus difficult to separate. Most analyses described in scientific literature obtained minimal separation of peaks and typically lack baseline separation. Our old method had zero separation of these peak.



Method adjustments tested:

To facilitate separation we:

- increased flow/pressures by .15ml/min from time of injection for 5 minutes.
- applied a reverse gradient starting at sample injection time of 70:30 ACN: H_20 to 55:45 ACN: H_2O.
- Explored Methanol: H_2O mixtures as well as MEOH inclusion as a 3rd solvent
- Investigated Tetrahydrofuran (THF) as a 3rd constituent by adding 15% THF to H_2O. Reverse gradient proved unsuccessful.

Results

- flow rate and gradient changes were moderately successful in peak separation. (table 1)
- Holding isocratic at higher flow for the first five minutes caused partial peak separation.(fig 1)
- Reverse gradient applied was unsuccessful.
- MeOh addition showed no improvements and Increased retention times (K') of all peaks as well as pressures.
- THF Improved peak narrowing but caused co-elution of previously separated peaks As well as a slight increase peak tailing for later compounds.

Conclusion

- Although baseline separation is not complete, enough separation was achieved to quantify acrolein and acetone individually. (Fig 1)

LC Parameters **Table 1**

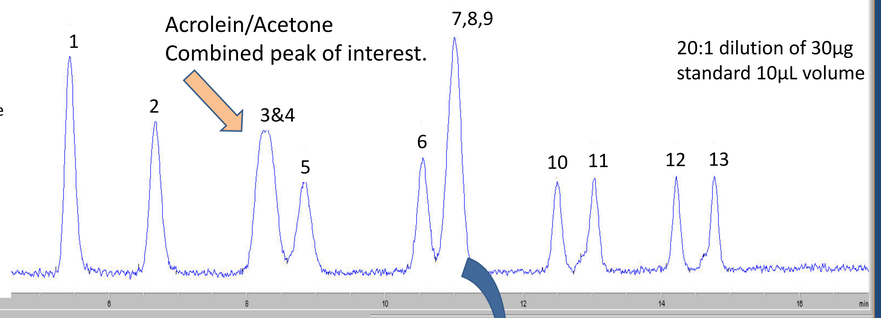
Separation of CARB Carbonyl-DNPH mix 1 & Method 1004 DNPH Mix 1

Column:	Restek Ultra AQ C18 (250mm X 4.6mm, 5µm particle size)
Sample injection volume:	10 & 20 µL

		Mobile phase		
Gradient:	Time(min)	% H2O	% ACN	µL/min
pre-run	-0.00	40	60	1.5
injection	0.00	30	70	1.65
	5.00	30	70	1.65
	5.01			1.50
	6.00	45	55	1.50
	7.00	40	60	1.50
	20.0	0	100	1.50
	21.0	60	40	1.50

Chromatogram BEFORE modification of method

1. Formaldehyde
2. Acetaldehyde
3. Acrolein
4. Acetone
5. Propionaldehyde
6. Crotonaldehyde
7. Methacrolein
8. 2-butanone
9. Butyraldehyde
10. Benzaldehyde
11. Valeraldehyde
12. m-Tolualdehyde
13. Hexaldehyde



Chromatogram AFTER modification of method

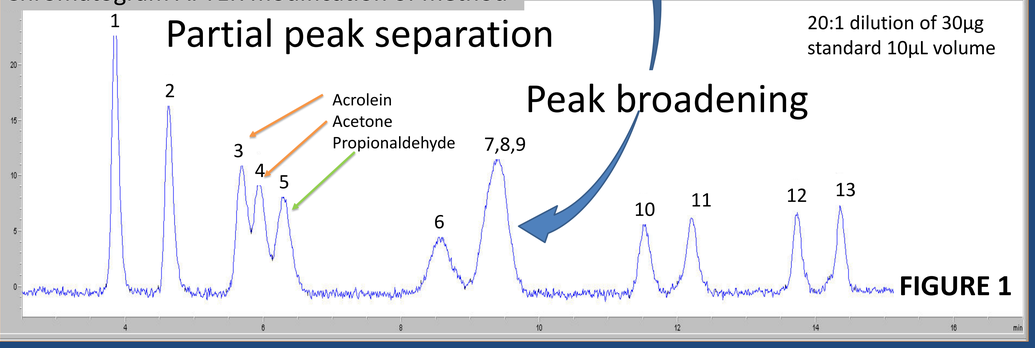


FIGURE 1

Detection limit

methods

- Detection limits were calculated as three times the standard deviation of replicate samples with concentrations a little higher than the noise level of the instrument.

results

- Increasing the volume of standard from 10µL to 20µL did not significantly impact the detection limit.(FIG 2&3)
- Detection limits were marginally, but not significantly higher for the first five compounds and varied for later eluting compounds.
- Reducing the volume of compounds may have increased baseline separation(the valleys between peaks) but did not impact the signal to noise ratio for detection limits as expected. More signal did not decrease variability.

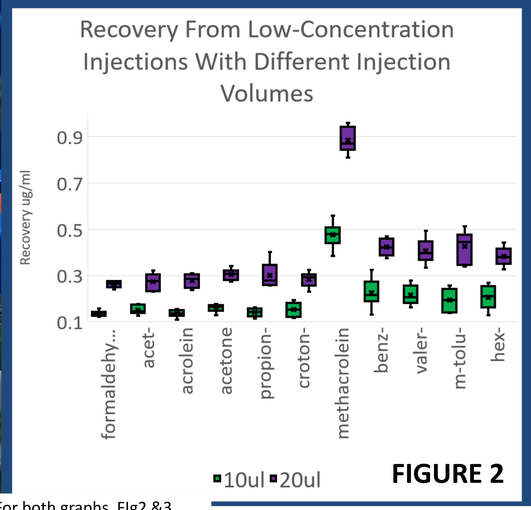
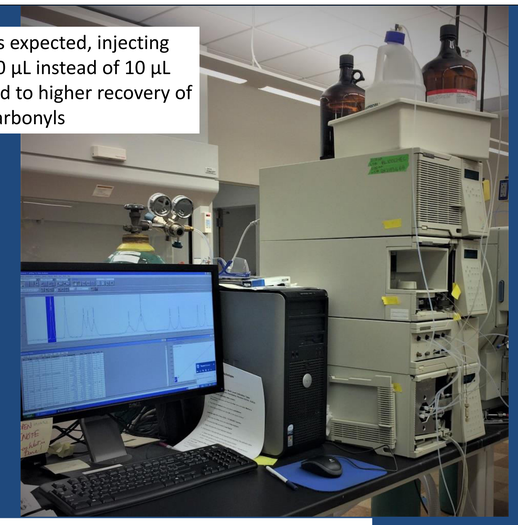


FIGURE 2

For both graphs FIg2 &3
X = mean, --- = 50% quartile
T whisker = max data value,
⍰ whisker = min data value



As expected, injecting 20 µL instead of 10 µL led to higher recovery of carbonyls

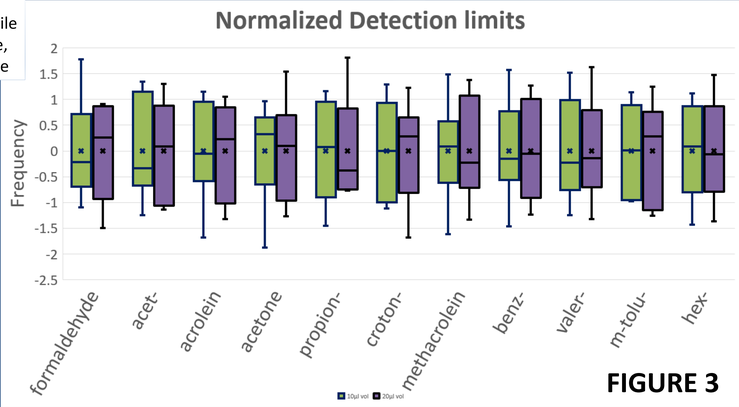


FIGURE 3

When we injected 20 µL instead of 10 µl the detection limit increase for some compounds and decreased for others. None of the changes were statistically significant.

What Still Needs to be Done?

Improvements to the method and hardware are the two primary areas where increased sensitivity and reduction of noise within the system is necessary:

- Work to separate out three target molecules that are currently unseparated; Methacrolein, 2-butanone, and butyraldehyde. (7,8,9) (Fig 1)
- Peak broadening may be mitigated using a smaller particle size within the column and increasing pressure through flow rate adjustments, as well as upgrading the analyzer with a new deuterium lamp.
- Further tests with THF may prove helpful in obtaining better baseline separation of peaks.

Acknowledgements

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abstract

- The Uintah Basin periodically experiences high ozone levels during the winter season. Carbonyl compounds are produced from various emission sources and are precursors to ground-level ozone production. We regularly measure carbonyl concentrations in air via high-performance liquid chromatography (HPLC) as part of our efforts to better understand and improve air quality in the Uintah Basin. For this project, we investigated improvements to our HPLC system looking to increase resolution, identification, and quantification of carbonyls in collected air samples. The goals of this work were to improve carbonyl compound separation and increase detection limits for our measurements. We investigated changes to flow rate/pressure, eluent composition, and sample injection volumes.